

Investigation of the influence of the time and deposition rate on the morphology and phase composition of calcium-phosphate coatings on a TiNi substrate

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In this work, the effect of technological modes of applying coatings based on calcium-phosphate powder on substrates of titanium nickelide alloys was studied. Compacted powders of calcium phosphates were used as targets. The optimal modes of coating deposition were selected and the deposition rates were determined. The structure, morphology, and phase composition of the alloy surfaces were studied by X-ray diffraction analysis and scanning electron microscopy. According to the results of the atomic force microscopy, a significant decrease in surface roughness was found depending on the thickness of the calcium-phosphate layer. When assessing the cytocompatibility of the alloys, a correlation was found between the number of viable mesenchymal stromal cell cultures and the thickness of the Ca – P coating. When the coating thickness increases, the viability coefficient of cell cultures increases either.

Key words: calcium phosphate, TiNi alloy, ion-plasma sputtering, microstructure, biocompatibility.

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1. Introduction

Titanium and titanium alloys are widely used as biomaterials and medical devices owing to their bioinertia characteristics [1–3]. The TiNi alloy, in particular, is one of the preferred implantation materials used for bone replacement. Despite its relatively good biological compatibility, monolithic TiNi alloys can hardly form a direct chemical bond with bone tissues; therefore, the surface modification is a decisive factor for the accelerated integration and increased biological activity of implants. The problem of obtaining biocompatible bioactive coatings intended for applying onto medical materials is sufficiently relevant and still unresolved. Coatings must be non-toxic, prevent from the diffusion of metal atoms and ions from the implant into the surrounding tissues, have high adhesion to the surface, and should not be abrasive in mobile elements. The coating must not elicit an immune response or be destroyed when interacting with living tissue.

The $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ hydroxyapatite (HAP) is a biocompatible material commonly used in medical coatings due to its composition that is similar to the principal component of the bone matrix [4]. Different methods are commonly used to apply thin hydroxyapatite coatings; these are electrochemical deposition [5–6], plasma spraying [7–8], spraying [9], immersion coating [10] and electrophoretic deposition [11]. In most cases when

forming apatite films, there are problems associated with subsequent high-temperature sintering, which can damage the metal substrate [12]. High-frequency magnetron sputtering, which has gained widespread use since the late 1990s, provides high adhesion strength between the base and coating materials and allows precise control of the coating composition [13, 14]. The phase composition of the mineral part of the bone tissue is diverse and contains various calcium-phosphate phases. Amorphous calcium phosphate, dicalcium phosphate dihydrate, octacalcium phosphate and tricalcium phosphate are used as bioactive coatings intended for implants used in reconstructive surgery [15]. Calcium phosphates are the main components of bones and, when used as a coating, they provide an optimal integration of implants into the bone. In vivo tests showed that the Ca – P coatings formed by means of ion-plasma spraying promote faster cell proliferation on the bone marrow surface compared to that of the implant free surface [10–12]. This result is achieved owing to the release of calcium and phosphate ions, which form an intermediate apatite layer required for adhesion, proliferation of osteogenic cells and the bone matrix formation.

This work is devoted to the choice of the mode of ion-plasma coating made of the calcium-phosphate powder intended for medical use onto titanium-nickelide alloy substrates and to the study of their morphology, elemental, phase composition and cytocompatibility.

2. Materials and methods

To spray coatings onto the substrate surface of titanium nickelide alloys, the method of plasma-assisted HF (high frequency) sputtering of powder targets applying a 200-mm diameter target without a magnetic system was used. This method was developed in the Laboratory of Plasma Emission Electronics (LPE) of the Institute of High Current Electronics (IHCE) of the Siberian Branch of the Russian Academy of Sciences (SB RAS). A “PINK” gas plasma generator was involved in intensifying the process of spraying the calcium-phosphate powder. The generator allowed creating the volumetric argon plasma in a working vacuum chamber. When the HF potential was fed to the target, argon ions were extracted from the plasma and bombarded the target, causing the intensive spraying of the calcium-phosphate powder. The technological process of coating consisted of several stages. The sample and the target for spraying were placed in the vacuum chamber. The air was pumped out up to a pressure of $5 \cdot 10^{-3}$ Pa. Argon was fed into the chamber to reach a pressure of 0.1–0.4 Pa; then the plasma generator was turned on and a negative bias was applied to the substrate for cleaning and activating the surface with argon plasma. After treating the surface, the HF generator connected to the target was turned on to initiate the process of spraying the material. The coating is formed on the substrate surface as a result of applying a bias voltage to it.

The volt-ampere characteristics of a nonindependent HF discharge were studied depending on the pressure of the working argon gas in the range from 0.1 to 0.5 Pa, the discharge current of the “PINK” plasma generator ranging from 5 to 70 A and the HF power fed to the target varying from 300 to 1000 W. On totality of the parameters, the argon pressure of 0.3 Pa, the discharge current of the “PINK” plasma generator equal to 30 A, and the HF power on the target of 600 W were chosen as the optimal parameters of the calcium-phosphate coating process.

In this mode, the rate of depositing the calcium-phosphate-based film is 0.5 $\mu\text{m}/\text{hour}$, while a stable operation of the “PINK” plasma generator is provided, without overheating the target, and a negative automatic bias on the target amounts to 800–1100 V, which is optimal for spraying the material and does not damage the HF input insulators.

To determine the optimal coating thickness, the processes of spraying lasting from 1 to 6 hours were carried out to obtain a coating thickness ranging from 500 nm to 1.8 μm . **Table 1** shows the coating thickness and the deposition rate depending on the process duration.

When increasing the deposition time beyond 4 hours up to 6 hours, there was not any observed increase in the coating thickness on the samples. The maximum obtained thickness of the coatings was 1.8 μm . Presumably, it was not possible to increase the thickness of the sprayed coatings because of spraying the coating with gas plasma.

The phase composition and structural parameters of the samples were studied using a Shimadzu XRD-6000 diffractometer (Shimadzu Corporation, Japan) in the

Table 1
Parameters of applying the Ca – P coatings on the surface of NiTi samples

| Sample No. | Deposition time, h | Thickness, μm | Deposition rate, $\mu\text{m}/\text{h}$ |
|------------|--------------------|--------------------------|---|
| 1 | 1 | 0.5 | 0.5 |
| 2 | 2 | 1 | 0.5 |
| 3 | 3 | 1.3 | 0.43 |
| 4 | 4 | 1.8 | 0.45 |

Cu K_{α} -radiation. The diffractograms were indexed by means of the PowderCell 2.4 full-profile analysis program and compared with the PDF 4+ database. The microstructure, morphology of the surface and the coating continuity were characterized using a scanning electron microscope Thermo Fisher Scientific Axia LoVac (Thermo Fisher Scientific Brno s.r.o., Czech Republic) along with a microanalyzer of the elemental composition. X-ray microfluorescence was performed by means of a Xenometrix X-Calibur spectrometer (Xenometrix LTD, Migdal HaEmek, Israel) applying a maximum voltage of 25 kV and a current of 10 μA in the helium atmosphere. A Nanoeducator scanning probe microscope (NT-MDT, Russia) equipped with a SOLVER HV vacuum chamber in a semi-contact mode was used to conduct the atomic-powered microscopic analysis. The data were processed by the Gwyddion 2.62 software. The cytocompatibility of the coatings was evaluated *in vitro* by MTT testing the culture viability of mesio-menchymal cells of the rat bone marrow after a 72-hour cultivation. Confocal laser scanning microscopy was performed on samples having a surface of 10×10 mm. The fluorescence localization of the matrix samples was recorded using a laser scanning confocal microscope LSM-750 (Carl Zeiss Microscopy, Germany).

3. Results and discussion

3.1. Structural studies of NiTi alloy samples coated with Ca – P

The X-ray structural analysis results allowed establishing the fact that all the samples mainly consisted of the NiTi phase in modifications of B2-austenite and B19'-martensite (**Fig. 1**). Structural reflexes of Ti_2Ni , Ni_3Ti and Ni_4Ti_3 compounds, being secondary phases in the Ti – Ni system, were also detected in the X-ray diffraction patterns. In addition to the Ti – Ni system reflexes, belonging to the substrate, the precision phase analysis allowed identifying the presence of low-intensity reflections of the coating phases from hydroxyapatite of the monoclinic syngony and beta-tricalcium phosphate of the hexagonal syngony. The coating phases are completely crystalline; No X-ray amorphous scattering was detected in the X-ray diffraction pattern in the area of structural reflections from the structural lines of hydroxyapatite and $\text{Ca}_3(\text{PO}_4)_2$ for all the alloys. An increase in the coating thickness from 0.5 μm to 1.8 μm allowed revealing an increase in the intensity of reflexes during diffraction from the planes of the crystal structures of hydroxyapatite and beta-tricalcium phosphate, which indicates an increase in their volume fraction.

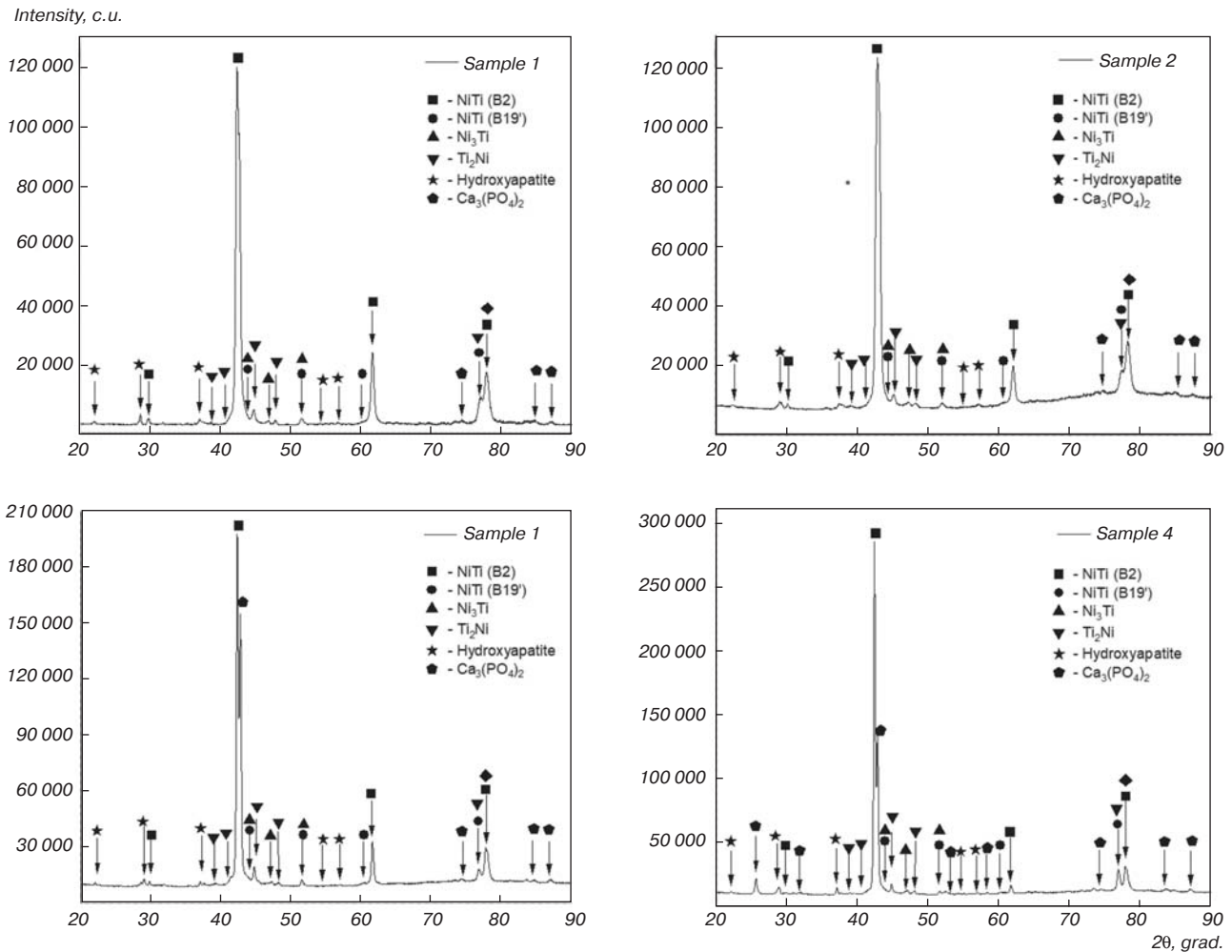


Fig. 1. X-ray diffraction patterns of the NiTi alloy samples covered with the Ca – P-base coating having the following thickness: sample 1 – 0.5 μm ; sample 2 – 1.0 μm ; sample 3 – 1.3 μm ; sample 4 – 1.8 μm

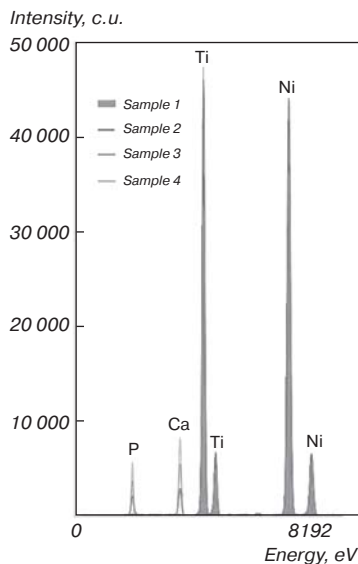


Fig. 2. Energy-dispersive spectrum of the NiTi alloy samples coated with Ca – P of various thicknesses

The X-ray fluorescence microanalysis of the surface of the NiTi alloy samples coated with Ca – P of different thicknesses demonstrated the presence of chemical Ti, Ni, Ca, P elements (**Fig. 2**). A decrease in the intensities of Ti and Ni reflexes from the substrate, as well as an increase in the intensities of Ca and P elements along with a coating thickness increase, was found. This is related to the fact that thin heterogeneous coatings were formed on the surface of samples 1 and 2 (**Fig. 3**), and the NiTi substrate can be viewed in the local areas, whereas a denser and more uniform calcium-phosphate layer was formed on samples 3 and 4 without visible defects (**Fig. 4**).

Dark inclusions ranging in size from 0.5 to 2 μm are visible on the surface of sample 1, which consist mainly of light elements such as oxygen, calcium and phosphorus. Small craters ranging in size from 1 to 2 μm can also be traced on the surface; they have a brighter glow and consist of heavy elements, such as titanium and nickel, being the NiTi substrate (**Fig. 3**). A uniform calcium-phosphate coating without visible defects was formed on sample 4 (**Fig. 4**). In addition, spherical bodies of 1–2 μm and conglomerates that are larger than 2 μm are present on the surface. The concentration distribution of the elements

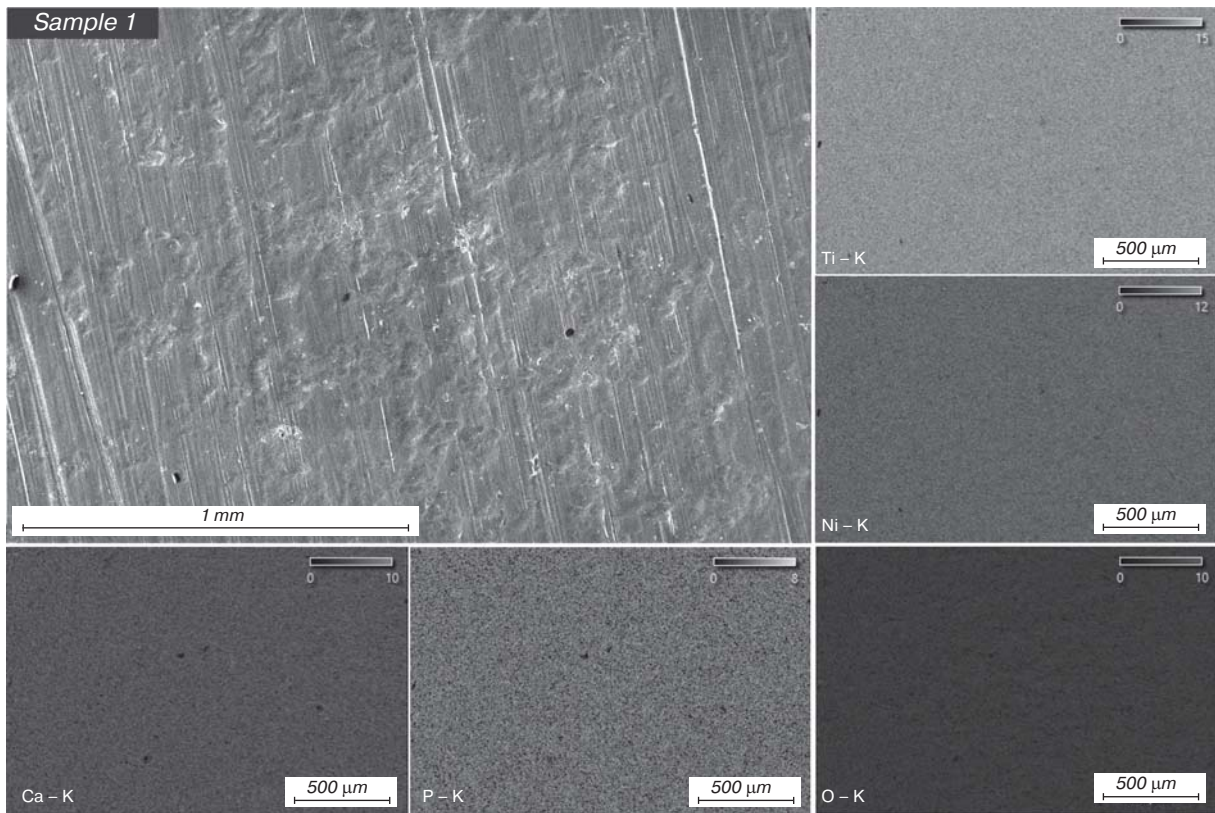


Fig. 3. Raster image of the microstructure and mapping by the chemical elements of sample 1 of the NiTi alloy coated with Ca – P having a thickness of 0.5 μm

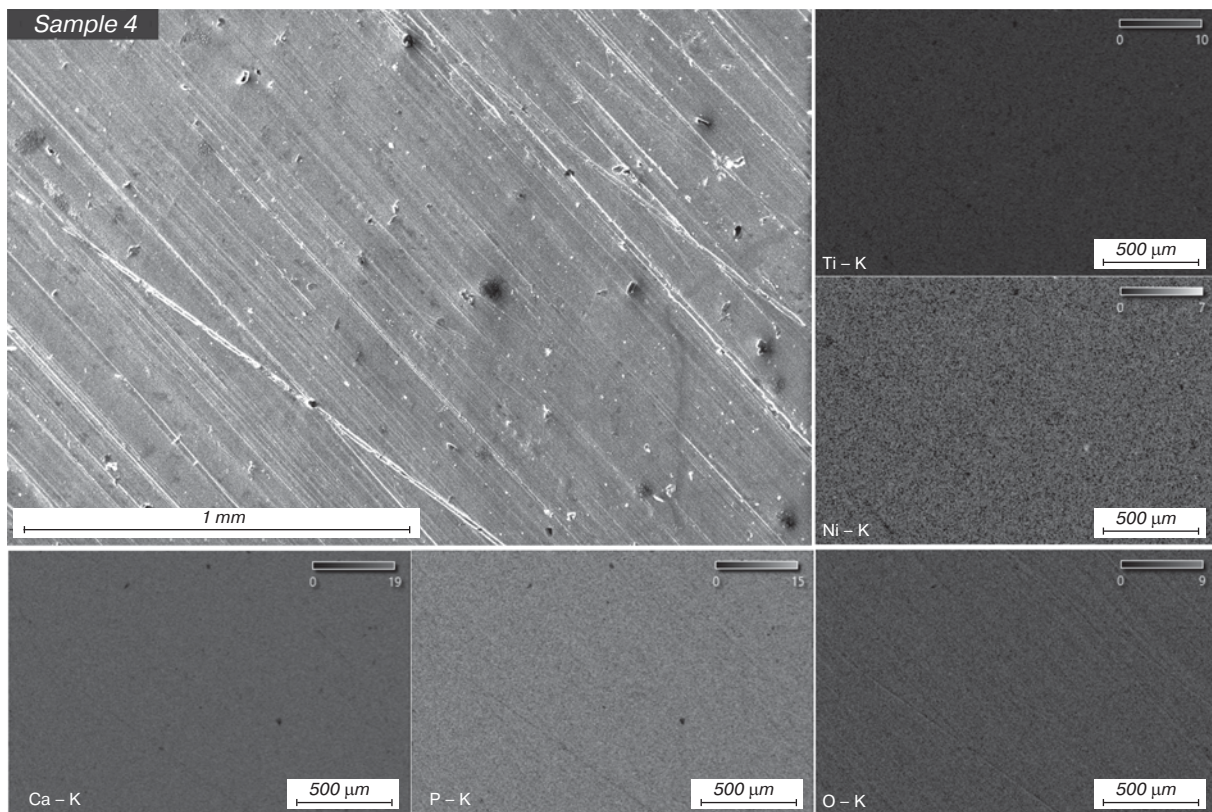


Fig. 4. Raster image of the microstructure and mapping by the chemical elements of sample 4 of the NiTi alloy coated with Ca – P having a thickness of 1.8 μm

Table 2
Concentration of the elements on the surface of the NiTi alloy samples coated with Ca – P of different thicknesses

| Elements | Sample 1, at. % | Sample 2, at. % | Sample 3, at. % | Sample 4, at. % |
|----------|-----------------|-----------------|-----------------|-----------------|
| O | 43.2 | 49.4 | 48.7 | 50.4 |
| P | 2.4 | 3.1 | 5.4 | 7.2 |
| Ca | 4.7 | 6.5 | 12.7 | 16.9 |
| Ti | 16.5 | 11.8 | 8.2 | 3.5 |
| Ni | 15.8 | 11.0 | 7.6 | 3.1 |

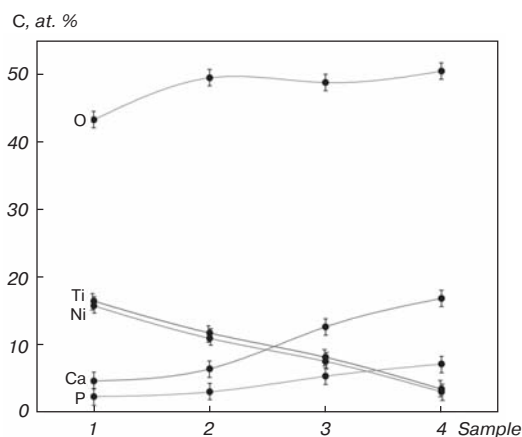


Fig. 5. Element dependence on the thickness of the layer on the surface of the NiTi samples coated with Ca – P of various thicknesses

Table 3
Surface relief parameters of the NiTi alloy coated with Ca – P of various thicknesses

| Sample No. | Coating thickness, μm | Roughness, nm | Level difference, μm |
|------------|----------------------------------|---------------|---------------------------------|
| 1 | 0.5 | 7.5 | 0.12 |
| 2 | 1 | 7.1 | 0.17 |
| 3 | 1.3 | 6.2 | 0.23 |
| 4 | 1.8 | 3.8 | 0.27 |

is more homogeneous; this indicates the formation of a dense calcium phosphate layer on the NiTi substrate. The quantitative distribution of the elements over the surface of the NiTi alloy samples coated with Ca – P is shown graphically in Fig. 5 and in Table 2. The EDS analysis data correlate with the XPA results. A decrease in the number of Ti and Ni elements, as well as a growth of the amount of Ca and P due to the coating thickness increase, was revealed.

Table 3 shows the surface relief parameters calculated by means of the AFM SOLVER HV device. The surface topography of the samples coated with Ca – P is determined mainly by the island globular relief typical of the HAP and Ca – P coatings (Fig. 6). The islands are formed by agglomerations of crystallites of calcium-phosphate phases. An increase in the thickness of the coatings increases the level difference, but at the same time it decreases the coating surface roughness.

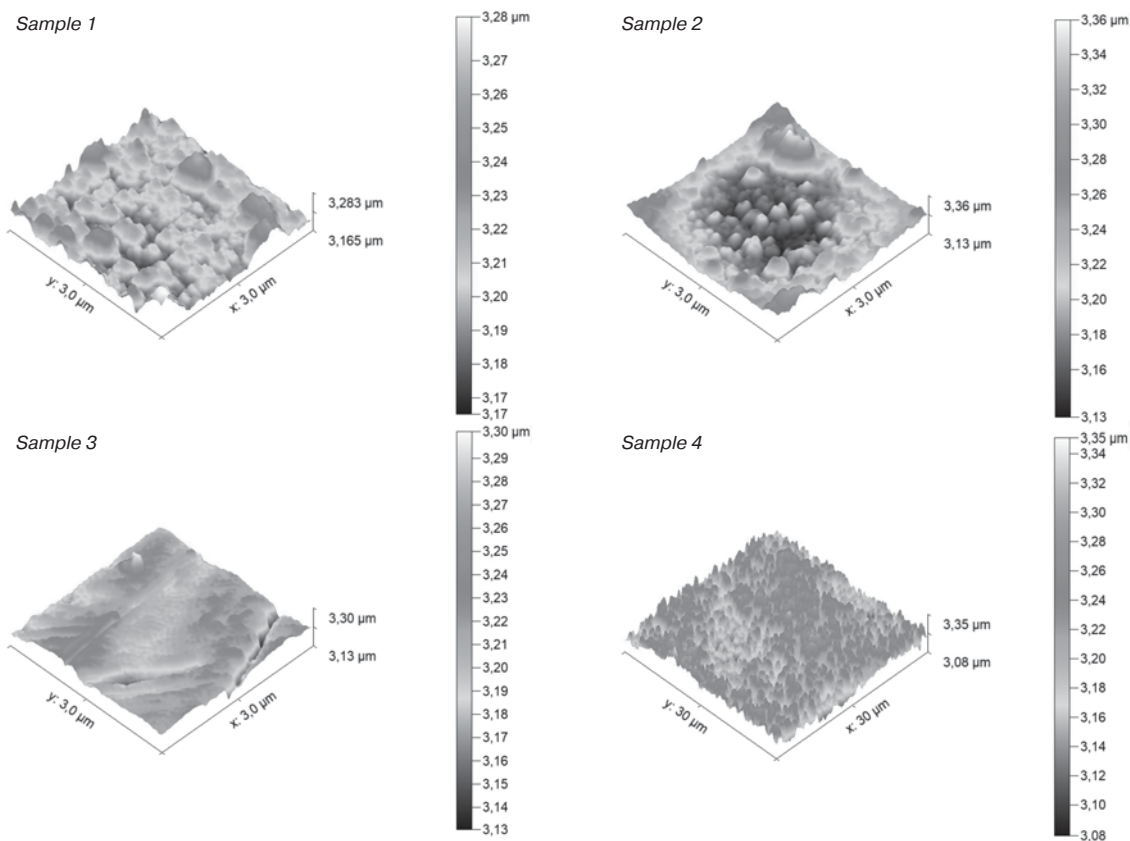


Fig. 6. AFM-images of the surface of the NiTi alloy coated with Ca – P of different thicknesses

Low values of the surface roughness of the NiTi alloy coated with Ca – P having a thickness of 1.8 μm within the scanned area are repeated for different sections of the sample, which indicates uniformity and density.

3.2. Cytocompatibility of the NiTi alloy samples coated with Ca – P

Cytocompatibility of the NiTi alloy samples coated with Ca – P of various thicknesses was evaluated *in vitro* using the MTT-test for the viability of mesenchymal stromal cell cultures after 72 hours of cultivation. The cell viability characteristic was used as a partial criterion for the material biocompatibility. The coefficient of decrease of viable cells during 72 hours per unit volume of the cell culture suspension under cytotoxic influence was chosen as a quantitative viability indicator. Freshly isolated mesenchymal stromal target cells of SVA mice in the amount of $2 \cdot 10^5$ cells per well plate were incubated along with the samples of TiNi alloys coated with Ca – P of different thicknesses to conduct the cytotoxic test. The number of viable cells was calculated as an arithmetic mean in 3 or 5 visual fields of the confocal microscope. The initial viability of the cells was at least 90%. The incubation exposure was selected to be within 72 hours, whereupon the viable cells were counted and their viability was calculated relatively the initial number of the cells separately for each plate, using the MTT test and microscopic observation in the Goryaev chamber.

The minimum number of viable flattened cells was noted on the surfaces of the TiNi alloy samples coated with Ca – P having a thickness of 0.5 μm after 72 hours of cultivation. A thin calcium-phosphate layer on the titanium nickelide surface did not provide an appropriate adhesion for cell lines, which led to cell death. A correlation was found between the number of viable mesenchymal stromal cell cultures and the Ca – P coating thickness. A coating thickness increase entailed an increase of the viability coefficient of the cell cultures. The largest number of the cells was attached to the surface of the TiNi alloys having the coating 1.8- μm thick, retaining their viability and high proliferative activity after 72 hours (Figs. 7–8).

4. Conclusion

As a result of the conducted studies, the ion-plasma sputtering modes for powder targets based on calcium-phosphates were established to obtain calcium-phosphate coatings on TiNi substrates 0.5–1.8- μm thick. The results of the structural studies of the surface by different methods can be combined favorably with each other, which indicates the reliability of the obtained results. The coating thickness was found not to influence the qualitative elemental and phase composition. All the coatings predominantly consist of the major crystalline phase of beta-tricalcium phosphate of the hexagonal syngony admixed with hydroxyapatite of the monoclinic syngony. An increase in the coating thickness ranging from 0.5 μm

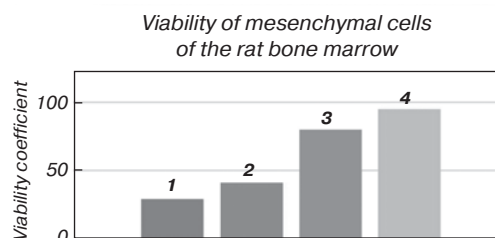


Fig. 7. Viability coefficient of mesenchymal cells of the rat bone marrow on the surface of the NiTi alloy samples coated with Ca – P of various thicknesses:

1 – sample with a 0.5- μm coating; 2 – sample with a 1.0- μm coating; 3 – sample with a 1.3- μm coating; 4 – sample with a 1.8- μm coating

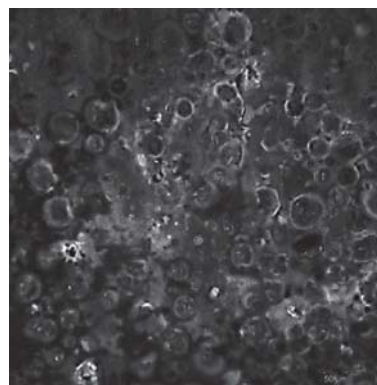


Fig. 8. Appearance of viable mesenchymal cells of the rat bone marrow on the surface of the NiTi alloy sample coated with Ca – P 1.8- μm thick

to 1.8 μm entails a volume fraction growth of the phases of hydroxyapatite and beta-tricalcium phosphate. A surface roughness decrease of the coatings was noted to vary from 7.5 to 3.8 nm along with an increase in the surface layer thickness from 0.5 to 1.8 μm . The coating surface cytocompatibility was shown to be determined not by roughness, but by the uniformity and thickness of Ca – P-based coatings. The best surface cytocompatibility was demonstrated by the samples having the Ca – P coating 1.8- μm thick. A homogeneous thick calcium-phosphate layer on the titanium nickelide surface, having no visible defects and delamination, provided a high viability of the cell cultures in 72 hours after incubation as compared to other samples.

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